0031-6997/06/5801-46–57$7.00
PHARMACOLOGICAL REVIEWS
Vol. 58, No. 1
60105/3091931
Printed in U.S.A
Copyright © 2006 by The American Society for Pharmacology and Experimental Therapeutics
Pharmacol Rev 58:46–57, 2006

PHARM REV

Orexins in the Regulation of the Hypothalamic-Pituitary-Adrenal Axis

RAFFAELLA SPINAZZI, PAOLA G. ANDREIS, GIAN PAOLO ROSSI, AND GASTONE G. NUSSDORFER

Departments of Human Anatomy and Physiology (R.S., P.G.A., G.G.N.) and Clinical and Experimental Medicine (G.P.R.), School of Medicine, University of Padua, Padua, Italy

Address correspondence to: Dr. Gastone G. Nussdorfer, Department of Human Anatomy and Physiology, Section of Anatomy, Via Gabelli 65, I-35121 Padova, Italy. E-mail: gastone.nusdorfer@unipd.it

Article, publication date, and citation information can be found at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.58.1.4.

PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

Abstract——**Orexin-A and orexin-B are hypothalamic peptides that act via two G protein-coupled receptors, named orexin type 1 and type 2 receptors (OX1-Rs and OX2-Rs). The most studied biological functions of orexins are the central control of feeding and sleep, but in the past few years findings that orexin system modulates the hypothalamic-pituitary-adrenal (HPA) axis, acting on both its central and peripheral branches, have accumulated. Orexins and their receptors are expressed in the hypothalamic paraventricular nucleus and median eminence and orexin receptors in pituitary corticotropes, adrenal cortex, and medulla. Whereas the effects of orexins on adrenal aldosterone secretion are doubtful, compelling evidence indicates that these peptides enhance glucocorticoid production in rats and humans. This effect involves a 2-fold mechanism: 1) stimulation of the adrenocorticotropin-releasing hormone-mediated pituitary release of adrenocorticotropin, which in turn raises adrenal glucocorticoid secretion; and 2) direct stimulation of adrenocortical cells via OX1-Rs coupled to the adenylate cyclase-dependent cascade. The effects of orexins on catecholamine release from adrenal medulla are unclear and probably of minor relevance, but there are indications that orexins can stimulate in vitro secretion of human pheochromocytoma cells via OX2-Rs coupled to the phospholipase C-dependent cascade. Evidence is also available that orexins enhance the growth in vitro of adrenocortical cells, mainly acting via OX2-Rs. Moreover, findings suggest that the orexin system may favor HPA axis responses to stresses and play a role in the pathophysiology of cortisol-secreting adrenal adenomas.**

I. Introduction

Orexins A and B are neuropeptides that were isolated from the rat hypothalamus by two independent groups of investigators in 1998 (De Lecea et al., 1998; Sakurai et al., 1998). They were originally named hypocretins because of their hypothalamic localization and their supposed similarity to secretin. Thereafter, their names was changed to orexins on the basis of their predominant expression in the hypothalamic feeding centers and their involvement in the control of food intake. Simultaneously with the discovery of orexins, two G protein-coupled receptors for these endogenous ligands have been identified by the same groups of investigators and called orexin type 1 and type 2 receptors (OX1-Rs[1] and OX2-Rs). Subsequent investigations showed that the orexin system also plays a role in the regulation of sleep/wakefulness, because orexin gene knockout mice and dogs with nonfunctional OX2-Rs because of a gene mutation display a narcolepsy-like condition (Chemelli et al., 1999; Lin et al., 1999), and narcoleptic humans lack orexin-containing neurons in the hypothalamus and orexins in the cerebrospinal fluid (Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000). Several excellent reviews (Meier, 1998; Wolf, 1998; De Lecea and Sutcliffe, 1999; Sakurai, 1999, 2005; Samson and Resch, 2000; Hungs and Mignot, 2001; Mignot, 2001; Siegel et al., 2001; Taheri and Bloom, 2001; Willie et al., 2001; Beuckmann and Yanagisawa, 2002; Kukkonen et al., 2002; Ferguson and Samson, 2003) on the involvement of the orexin system in the regulation of feeding and sleep are available.

Further studies suggested a possible role of the orexin system in the central stimulation of autonomic functions, including regulation of blood pressure and heart rate and neuroendocrine axes, as the hypothalamic-pituitary-adrenal (HPA) axis (reviewed by Samson and Resch, 2000; Taheri and Bloom, 2001; Kukkonen et al., 2002; Ferguson and Samson, 2003; Taylor and Samson, 2003; Jöhren et al., 2004). This last contention is of great interest, because compelling evidence has been accumulated that neuropeptides that are involved in the central regulation of feeding [e.g., leptin, neuropeptide Y (NPY), neuropeptide W, and cholecystokinin] also control the central branch of the HPA axis (Crawley and Corwin, 1994; Krysiak et al., 1999; Ahima and Flier, 2000; Wauters et al., 2000; Baker et al., 2003; Malendowicz et al., 2003). Moreover, such peptides, acting via specific receptors, have been also found to exert direct regulatory control of the peripheral branch of the HPA axis (Bornstein et al., 1997; Glasow et al., 1998; Pralong et al., 1998; Glasow and Bornstein, 2000; Malendowicz et al., 2001c; Renshaw and Hinson, 2001; Hochól et al., 2004; Mazzocchi et al., 2004, 2005; Nussdorfer et al., 2005; Spinazzi et al., 2005a). Thus, after a brief account of the biology of orexin system, we will herein survey findings indicating that orexins, like the other peptides modulating food intake, are involved in the functional regulation of the adrenal gland under physiological and pathological conditions.

[1] Abbreviations: OX1-R, orexin type 1 receptor; OX2-R, orexin type 2 receptor; HPA, hypothalamic-pituitary-adrenal; NPY, neuropeptide Y; pp, prepro; CHO, Chinese hamster ovary; PLC, phospholipase C; IP_3, inositol triphosphate; PK, protein kinase; AC, adenylate cyclase; ICC, immunocytochemistry; PVN, paraventricular nucleus; RT, reverse transcription; PCR, polymerase chain reaction; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; AVP, arginine-vasopressin; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis; SQ-22536, 9-(tetrahydro-2′-furyl)adenine; H89, *N*-[2-(4-bromocinnamylamino)ethyl]-5-isoquinoline; U-73122, 1-[6-[[17β-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; MAPK, mitogen-activated protein kinase; PD98059, 2′-amino-3′-methoxyflavone; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; SB-334867, 1-(2-methylylbenzoxanzol-6-yl)-3-(1,5)naphthyridin-4-yl-urea hydrochloride.

II. Biology of Orexins and Their Receptors

A. Orexin Biosynthesis

Orexins derive from the post-translational cleavage of a 130-131 amino acid precursor, the prepro (pp)-orexin, that displays an 83% amino acid identity between humans and rats. The human pp-orexin gene is located on chromosome 17q and consists of only two exons and one intron (Sakurai et al., 1999). After detachment of the

PHARM REV
PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

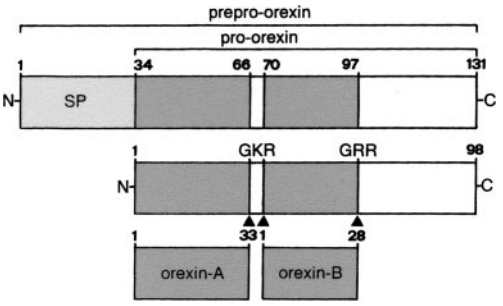

FIG. 1. Scheme illustrating the post-translational processing of pp-orexin. The arrowheads indicate the sites where, after removal of signal peptide (SP), pro-orexin is cleaved by prohormone convertase at sites with basic amino acid residues (GKR and GRR) to produce mature orexin-A and orexin-B.

N-terminal 33-amino acid residue signal peptide, pp-orexin (now pro-orexin) is cleaved by prohormone convertases to yield one molecule each of orexin-A and orexin-B (Fig. 1).

Orexin-A is a 33-amino acid peptide of ~3.5 kDa, which possesses an N-terminal pyroglutamyl residue, a C-terminal amidation, and two intrachain disulfide bridges between adjacent cysteine residues in the 6 and 12 and 7 and 14 positions (Fig. 2). The amino acid sequence of orexin-A is well preserved among mammalian species (Sakurai et al., 1998; Dyer et al., 1999). Mammalian orexin-B is a 28-amino acid peptide of ~2.9 kDa with C-terminal amidation. It is well preserved among mammalian species, and displays substantial identity in amino acid sequence of the C terminus with orexin-A (Fig. 2). Orexin-A is much more stable than orexin-B (Kastin and Akerstrom, 1999), which explains why its tissue and blood concentrations are markedly higher than those of orexin-B. Moreover, orexin-A displays higher liposolubility than orexin-B, which makes it, in contrast with orexin-B, blood-brain barrier permeant (Kastin and Akerstrom, 1999).

B. Orexin Receptors and Their Signaling Mechanisms

OX1-Rs and OX2-Rs are seven transmembrane domain G protein-coupled receptors that are encoded by two genes located on chromosomes 1 and 6, respectively (Sakurai et al., 1998). Human OX1-Rs and OX2-Rs are 425 and 444 amino acids long, respectively, possess a 64% amino acid sequence identity, and are highly conserved among mammalian species (94% and 95% amino acid identity for OX1-Rs and OX2-Rs between humans and rats). The OX1-R is selective for orexin-A, whereas the OX2-R is nonselective for both orexins: affinities (EC_{50}) of orexin-A for OX1-Rs and OX2-Rs are 30 and 34 nM, and those of orexin-B are 2500 and 60 nM, respectively (De Lecea et al., 1998; Sakurai et al., 1998).

The bulk of evidence, obtained in a Chinese hamster ovary (CHO) cell line stably expressing OX1-Rs and/or OX2-Rs, indicates that activation of both receptors increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Smart et al., 1999; Kane et al., 2000; Lund et al., 2000; Holmqvist et al., 2001; Ammoun et al., 2003; Larsson et al., 2005). This effect ensues from the activation of phospholipase C (PLC), which in turn catalyzes the break-

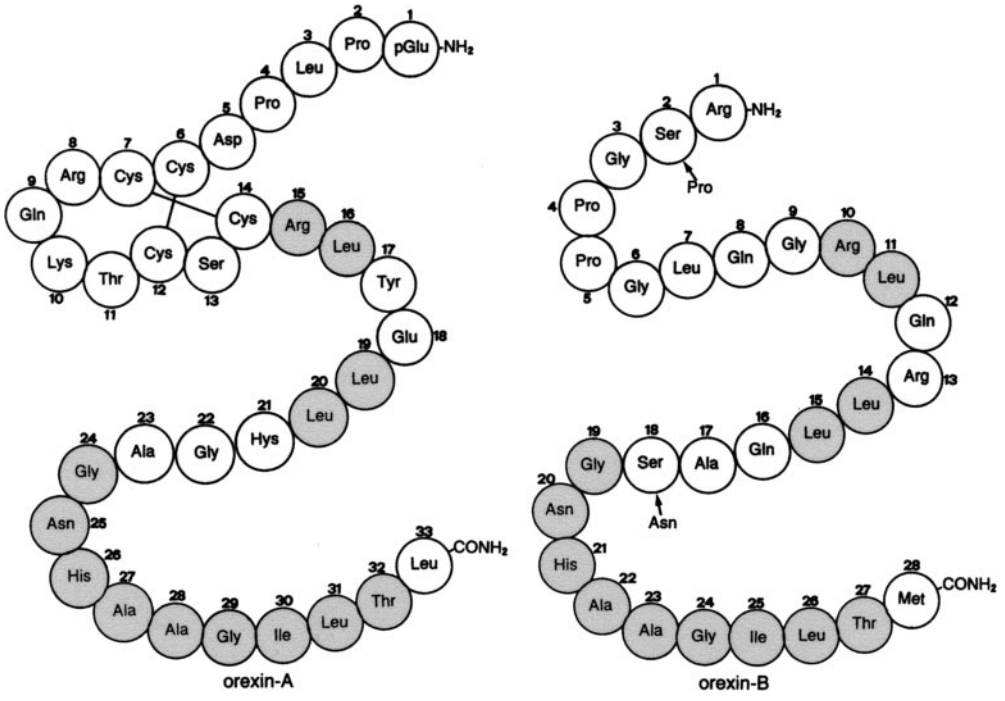

FIG. 2. Amino acid sequence of human orexin-A and orexin-B. Orexin-A possesses an N-terminal pyroglutamyl residue and two intramolecular disulfide bridges between cysteine residues in 6 and 12 and 7 and 14 positions. The orexin-A amino acid sequence is identical in humans, sheep, pigs, rats, and mice. Human orexin-B differs from that of pigs, rats, and mice for two amino acid substitutions in 2 and 18 positions (arrows). Identical amino acids between orexin-A and orexin-B are shadowed.

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

down of phosphatidylinositol to inositol triphosphate (IP_3) and diacylglycerol. Diacylglycerol activates protein kinase (PK) C and IP_3 enhances Ca^{2+} release from intracellular stores that in turn activates PKC. PKC and the increased $[Ca^{2+}]_i$ open voltage-gated Ca^{2+} channels, thereby stimulating Ca^{2+} influx and inducing cell membrane depolarization (Fig. 3). These effects of OX1-Rs and OX2-Rs are obtained by their coupling to excitatory G protein Gq/11. Findings indicate that OX2-Rs coupling to inhibitory G protein G_i/G_o may also cause K^+ efflux in neurons and cell membrane hyperpolarization (Fig. 3) (Willie et al., 2001; Beuckmann and Yanagisawa, 2002). It is also to be mentioned that there is evidence in human and rat adrenocortical cells that OX1-Rs are also coupled to the adenylate cyclase (AC)/PKA signaling pathway (see sections IV.B.2. and V.B.2.). Accordingly, the presence of putative phosphorylation sites for PKA has been reported in both OX1-Rs and OX2-Rs (Kreegipuu et al., 1999; Kukkonen et al., 2002), and OX1-Rs were found to stimulate AC in CHO cells via a low potency Gs coupling and a high potency PLC/PKC coupling (Holmqvist et al., 2005).

III. Expression and Function of Orexins and Their Receptors in the Central Branch of the Hypothalamic-Pituitary-Adrenal Axis

A. Expression of Orexins and Their Receptors

1. Hypothalamus. Orexins and their receptors were originally discovered in the rat and human brain. The huge mass of investigations dealing with the detection of pp-orexin and orexin receptor mRNA in the total hypothalamus homogenates have already been extensively reviewed (see section I.). Therefore, we will herein restrict discussion to survey studies on the hypothalamic distributions of orexin-positive neurons and OX1-Rs and OX2-Rs.

a. Orexin expression. In situ hybridization showed that pp-orexin mRNA expressing neurons were mainly present in the lateral hypothalamic area of both adult (Lopez et al., 2000, 2001; Stricker-Krongrad and Beck, 2002) and newborn rats, in which expression increased between 15 and 20 days of age (Yamamoto et al., 2000). Immunocytochemistry (ICC) confirmed the main localization of orexin-positive neurons in the rat lateral hypothalamic area, especially at the level of the median eminence, with extensions to the perifornical nucleus and posterior hypothalamic area. From these neurons fibers project widely to the brain (cortex, olfactory bulb, thalamus, and brainstem) and other hypothalamic nuclei, including the arcuate nucleus, supraoptic nucleus, and paraventricular nucleus (PVN) (Van den Pol et al., 1998; Chen et al., 1999; Cutler et al., 1999; Date et al., 1999; Nambu et al., 1999; Funahashi et al., 2000; Cai et al., 2001; Kanenishi et al., 2004). The presence of orexin-A- and orexin-B-positive neurons in the lateral hypothalamic area, with orexinergic fibers throughout the entire hypothalamus, has been also demonstrated by ICC in Syrian, Siberian, and Djungarian hamsters (McGranaghan and Piggins, 2001; Khorooshi and Klingenspor, 2005). Fasting- and insulin-induced hypoglycemia increased pp-orexin mRNA expression and orexin-A and orexin-B concentrations in the rat hypothalamus (Lopez et al., 2000; Cai et al., 2001; Karteris et al., 2005), and the i.c.v. administration of leptin, which lowers food intake and body weight, reversed the effects of fasting. By contrast, the i.p. injection of leptin was found to increase pp-orexin mRNA in the lateral hypothalamic area of newborn rats (Yamamoto et al., 2000). Adrenalectomy induced a 50% decrease in the pp-orexin mRNA in the rat hypothalamus, and dexamethasone administration abrogated this effect (Stricker-Krongrad and Beck, 2002). pp-Orexin mRNA has been shown to undergo a net rise during pregnancy in rats (Kanenishi et al., 2004), and the concentrations of both orexin-A and orexin-B increased in proestrus, which could contribute to luteinizing hormone and prolactin surges (Porkka-Heiskanen et al., 2004). Finally, an age-related (from 3 to 8 months) decrease in orexin-A, but not orexin-B,

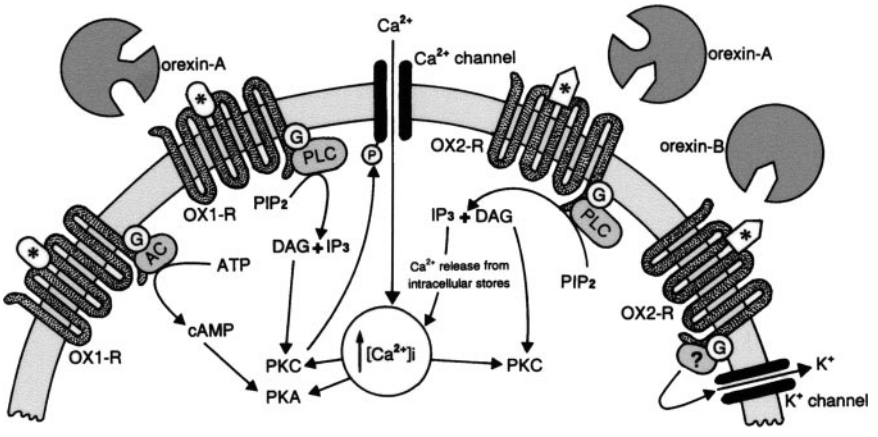

FIG. 3. Schematic drawing of the main signaling pathways of OX1-Rs and OX2-Rs upon activation by orexin-A and orexin-A or orexin-B, respectively. G, G protein; P, phosphorylation site; PIP2, phosphatidylinositol biphosphate. Other abbreviations are indicated in the text. Asterisks mark the orexin-recognizing sites of OX1-Rs and OX2-Rs.

PHARM REV
PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

concentration in the rat hypothalamus has been demonstrated (Porkka-Heiskanen et al., 2004).

b. Orexin receptor expression. In situ hybridization and ICC showed that in the rat OX1-R expression was largely restricted to the ventromedial and dorsomedial nuclei and OX2-R expression to the lateral hypothalamic area, PVN, and arcuate, mammillary and tuberomammillary nuclei (Trivedi et al., 1998; Lu et al., 2000; Marcus et al., 2001). Cluderay et al. (2002) confirmed the distribution of the OX2-Rs but were unable to detect the expression of OX1-R mRNA. In contrast, Hervieu et al. (2001) did not find OX2-R expression but reported the presence of OX1-R mRNA in the above-mentioned nuclei expressing the OX2-R. Overall, these conflicting findings cast doubts about the specificity of the probes used. In situ hybridization demonstrated that the hypothalamic distribution of orexin receptors in the male sheep reflected that occurring in the rat, with the following exceptions: OX1-R mRNA was present in the lateral hypothalamic area, arcuate nucleus, and PVN and both OX1-R and OX2-R mRNA in the median eminence (Zhang et al., 2005). Food deprivation has been reported to up-regulate the expression of OX1-Rs and OX2-Rs in the rat hypothalamus, as well as to increase their coupling to the AC- and PLC-dependent cascades (Karteris et al., 2005).

2. Pituitary Gland. No expression of orexins, as mRNA and proteins, was found in the human pituitary gland (Arihara et al., 2000). Likewise, there is evidence that this may occur in the gland of other mammalian species.

Reverse transcription (RT)-polymerase chain reaction (PCR) and ICC demonstrated the expression of both OX1-Rs and OX2-Rs in the pituitary gland of rats (Date et al., 2000; Jöhren et al., 2001, 2003), sheep (Zhang et al., 2005), and humans (Blanco et al., 2001). According to Blanco et al. (2001), the OX1-R was present in acidophils, where it was coexpressed with GH, and the OX2-R in the pars intermedia and basophils, where it was coexpressed with ACTH. In the rat pituitary, OX1-R expression prevailed over that of OX2-R and was higher in males than in females (Jöhren et al., 2001). Gonadectomy increased OX1-R expression in both female and male rats, the effect being abrogated by the administration of 17β-estradiol and testosterone, respectively (Jöhren et al., 2003).

B. Effects of Orexins

The hypothalamic nuclei involved in the secretion of adrenocorticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) contain orexin-positive neurons and are provided with orexin receptors (see section III.A.1.), and pituitary gland possesses orexin receptors (see section III.A.2.). Here, we will survey findings indicating that orexin system plays a role in the control of AVP, CRH, and ACTH release, at least in the rat.

Orexin-A and orexin-B were found to enhance c-*fos* mRNA expression (Kuru et al., 2000), and orexin-A has been reported to raise CRH and AVP mRNA levels in the PVN (Al-Barazanji et al., 2001; Brunton and Russell, 2003). Orexin-A stimulated CRH release from hypothalamic explants (Russell et al., 2001), and its application to hypothalamic slices resulted in depolarization and an increase in spike frequency of PVN neurons (Samson et al., 2002). Moreover, the i.c.v. administration of orexin-A increased plasma AVP concentration in rabbits (Matsumura et al., 2001). The CRH-stimulating action of orexin-A appears to be, at least partly, mediated by NPY via the Y1 receptor subtype, because orexin-A increased not only CRH but also NPY release from hypothalamic explants and its stimulating effect on CRH release was blocked by an Y1 receptor antagonist (Russell et al., 2001). Accordingly, the orexin-induced surge in the blood level of corticosterone (see section IV.B.2.) was blocked by pretreatment with a NPY antagonist or NPY antiserum (Jaszberényi et al., 2001). However, more recent findings did not confirm the involvement of NPY in the orexin-induced stimulation of HPA axis in rats (Moreno et al., 2005). In fact, the destruction of the arcuate nucleus and hypothalamic NPY-ergic activity by neonatal monosodium L-glutamate treatment was found to impair food-intake response to i.c.v. orexin-A but to spare glucocorticoid response.

Orexins enhance the pituitary release of ACTH, as shown by the rapid rise in ACTH blood level after their i.c.v. administration (see section IV.B.2.), and the effect appears to be indirectly mediated by the stimulation of hypothalamic production of CRH and AVP, the main physiological ACTH secretagogues (reviewed in Engelmann et al., 2004). In fact, orexins neither affected basal ACTH release from cultured rat pituitary cells (Samson and Taylor, 2001) nor raised pro-opiomelanocortin mRNA expression in the pituitary gland (Al-Barazanji et al., 2001). However, orexin-A and orexin-B were found to prevent submaximally CRH-stimulated ACTH release by cultured pituitary cells, minimal and maximal effective concentrations being 10^{-10} and 10^{-7} M, respectively (Samson and Taylor, 2001). Orexin-A (10^{-7} M) did not affect either basal or CRH-stimulated cyclic adenosine 3′,5′ monophosphate (cAMP) production, and its inhibitory action on CRH-induced ACTH release was reversed by the PKC inhibitor calphostin C, but not pertuxis toxin. Samson and Taylor (2001) concluded that this effect of orexins is mediated by OX1-Rs coupled to the PKC-dependent cascade. It remains to be ascertained whether these findings are indicative of the existence of a local counterregulatory mechanism aimed at quenching an exceedingly high CRH-mediated ACTH response to orexins.

The findings reviewed above have been obtained in the rat, but indirect evidence suggests that the endogenous orexin system is involved in the central regulation of the HPA axis in humans also. In fact, narcoleptic

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

(orexin-deficient) patients displayed blunted ACTH and cortisol secretion (Kok et al., 2002).

IV. Expression and Function of Orexins and Their Receptors in the Peripheral Branch of the Hypothalamic-Pituitary-Adrenal Axis

A. Expression of Orexins and Their Receptors

1. Orexin Expression. Low pp-orexin mRNA expression has been detected by RT-PCR in human adrenal glands (Nakabayashi et al., 2003), and the presence of pp-orexin and orexin-A proteins has been demonstrated by Western blotting in both adult and fetal (2nd trimester) human glands (Karteris et al., 2001; Randeva et al., 2001), but more recent studies did not confirm these findings (Spinazzi et al., 2005b). No orexin expression was found in the rat adrenal glands (Lopez et al., 1999, 2001; Karteris et al., 2005). Collectively, these findings suggest that the activation of adrenal OX1-Rs and OX2-Rs could be mediated by circulating orexins (see section VI.).

2. Orexin Receptor Expression

a. Human adrenal gland. Blanco et al. (2002) reported ICC evidence that adrenal cortex and medulla were exclusively provided with OX1-Rs and OX2-Rs, respectively. In contrast, combined RT-PCR, fluorescent in situ hybridization, ICC, and Western blot findings consistently showed that adult and fetal adrenal cortex exclusively expressed the OX2-R (Karteris et al., 2001; Randeva et al., 2001). Mazzocchi et al. (2001b) reported rather different RT-PCR findings: both OX1-R and OX2-R mRNAs were expressed in zona fasciculata (ZF)-zona reticularis (ZR) and adrenal medulla and only OX1-R mRNA in zona glomerulosa (ZG). Subsequent studies confirmed the expression of both receptor subtypes in freshly dispersed and cultured human adrenocortical cells (Ziolkowska et al., 2005), and real-time PCR and Western blotting demonstrated that the levels of expression of OX1-Rs and OX2-Rs did not differ significantly (Spinazzi et al., 2005b).

b. Rat adrenal gland. RT-PCR allowed the detection of both OX1-R and OX2-R mRNAs in adrenal homogenates (Lopez et al., 1999, 2001; Malendowicz et al., 2001a) and freshly dispersed ZF/ZR cells (Spinazzi et al., 2005c). In contrast, no expression of OX1-Rs (Nanmoku et al., 2000) or very low expression of OX1-Rs compared with OX2-Rs have been reported (Jöhren et al., 2001, 2003). Of interest, fluorescent in situ hybridization showed OX2-R mRNA in the ZG and ZR, but not the ZF and adrenal medulla (Jöhren et al., 2001). OX2-R expression displayed an evident sex dimorphism, being higher in males than in females (Jöhren et al., 2001). Accordingly, ovariectomy increased it in females and orchiectomy decreased it in males, the effects being hampered by 17β-estradiol and testosterone replacement, respectively (Jöhren et al., 2003). In contrast with the hypothalamus (see section III.A.1.), food deprivation was found to down-regulate OX1-R and OX2-R mRNA expression in the rat adrenal cortex, as well as to markedly reduce orexin receptor coupling to G_q, G_s, and G_o and to increase coupling to G_i with the ensuing impairment of cAMP and IP_3 responses to orexin-A (Karteris et al., 2005). These data suggest that hypothalamic, but not adrenal orexin receptors may mediate the fasting-induced surge in glucocorticoid blood levels in rats (Dallman et al., 1999).

c. Sheep and pig adrenal gland. The sheep adrenal cortex was found to contain only OX1-R mRNA (Zhang et al., 2005). The exclusive expression of OX1-Rs has been also reported in cultured pig adrenocortical and adrenomedullary cells, the expression being exceedingly higher in the former than in the latter (Nanmoku et al., 2002).

B. Effects of Orexins

1. Aldosterone Secretion. Despite orexin receptors having been demonstrated in the ZG (see section IV.A.2.), the bulk of evidence indicates that orexins do not affect aldosterone secretion from dispersed rat and human ZG cells (Malendowicz et al., 1999a; Mazzocchi et al., 2001b). However, cultured pig adrenocortical cells (actually a mixture of ZG and ZF/ZR cells) displayed a sizable aldosterone response (60% increase) to 10^{-7}/10^{-6} M orexin-A (Nanmoku et al., 2002), but it must be recalled that the physiology of cultured cells may not completely reflect that of freshly dispersed cells.

In contrast, findings that orexin administration increases aldosterone plasma concentration in rats are available. Acute s.c. injection of orexins (10 nmol/kg) raised within 60 to 120 min the level of circulating aldosterone (Malendowicz et al., 1999b; Nowak et al., 2000), and chronic orexin administration (daily s.c. injections of 20 nmol/kg for 7 days) increased it by approximately 70% (Malendowicz et al., 2001a). Conceivably, the in vivo aldosterone secretagogue effect of orexins is indirectly mediated by their stimulating action on the hypothalamic release of CRH and AVP (see section III.B.), which not only stimulate pituitary ACTH secretion but also directly enhance the secretory activity of ZG cells (reviewed in Nussdorfer, 1996). Alternatively, it could be also possible that in vivo administration of orexins may alter plasma angiotensin II or K^+ levels.

2. Glucocorticoid Secretion. Compelling evidence indicates that orexins increase glucocorticoid secretion from inner adrenocortical layers, via a 2-fold mechanism: 1) stimulation of CRH/AVP-mediated release of ACTH (see section III.B.); and 2) direct stimulation of ZF/ZR cells, which are provided with orexin receptors (see section IV.A.2.).

a. Indirect mechanism. In vivo studies showed that the systemic administration of orexin-A acutely raised ACTH and corticosterone blood levels in the rat (Malendowicz et al., 1999b; Nowak et al., 2000). Orexin-B was ineffective, which accords well with the notion that

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

orexin-A rapidly crosses the blood-brain barrier by simple diffusion, whereas orexin-B is rapidly degraded in the blood (see section II.A.). Orexins are much more effective when they are administered i.c.v. Orexin-A was found to enhance both ACTH and corticosterone blood levels, with the corticosterone response being slightly delayed (Ida et al., 2000; Jaszberényi et al., 2000; Kuru et al., 2000; Al-Barazanji et al., 2001; Russell et al., 2001; Brunton and Russell, 2003). Orexin-B was less effective than orexin-A (Jaszberényi et al., 2000, 2001; Kuru et al., 2000), thereby suggesting that the effect was mediated by both OX1-Rs and OX2-Rs (see section II.B.). The ACTH and corticosterone responses to orexin-A were blunted in pregnant rats (Brunton and Russell, 2003), a finding not apparently in keeping with the reported enhanced expression of pp-orexin during pregnancy (see section III.A.1.). The contention that the in vivo glucocorticoid secretagogue action of orexins is indirectly mediated by their hypothalamic effects is confirmed by the demonstration that pretreatment with the CRH antagonist α-helical CRH(9-41) blocked orexin-A-evoked surges in ACTH and corticosterone plasma concentrations in rats (Ida et al., 2000; Samson et al., 2002).

b. Direct mechanism. The bulk of evidence indicates that orexins enhance glucocorticoid secretion acting via OX1-Rs located on ZF/ZR cells despite the prevalence of OX2-Rs in adrenal glands described by some groups of investigators (see section IV.A.2.). Although earlier studies showed that both orexins stimulate corticosterone production (Malendowicz et al., 1999a), subsequent investigations consistently showed that only orexin-A concentration dependently increased basal corticosterone or cortisol secretion from dispersed or cultured rat and human ZF/ZR cells (Mazzocchi et al., 2001b; Spinazzi et al., 2005b). Minimal and maximal effective concentrations were 10^{-8} and $10^{-7}/10^{-6}$ M in rats and 10^{-10} and 10^{-8} M in humans. The ineffectiveness of orexin-B, which exclusively binds OX2-Rs (see section II.B.), ruled out the involvement of this receptor subtype in the direct glucocorticoid secretagogue action of orexins. This contention has been directly supported by the demonstration that the blockade of OX1-Rs by specific antibodies abolished the secretory response of both cultured rat and human ZF/ZR cells to orexin-A, whereas the immunoneutralization of OX2-Rs was ineffective (Ziolkowska et al., 2005).

Orexin-A did not maximally affect ACTH-stimulated glucocorticoid secretion from dispersed ZF/ZR cells, but enhanced the glucocorticoid response to maximal effective concentrations of angiotensin II and endothelin-1 (Malendowicz et al., 1999a; Mazzocchi et al., 2001b), which are agonists mainly activating PLC/PKA cascade (reviewed in Nussdorfer et al., 1999). This finding suggests that orexin-A and ACTH share a common signaling mechanism, i.e., the activation of the AC-dependent cascade. This contention was supported by the demonstration that 1) orexin-A enhanced cAMP but not IP_3 production by ZF/ZR cells (Malendowicz et al., 1999a; Mazzocchi et al., 2001b) and 2) the glucocorticoid secretagogue effect of orexin-A was abolished by either the AC inhibitors SQ-22536 or the PKA inhibitor H89 and unaffected by the PLC and PKC inhibitors U-73122 and calphostin-C (Spinazzi et al., 2005b). Randeva et al. (2001) reported that orexin-A (from 10^{-11} to 10^{-7} M) increased not only cAMP, but also IP_3 production from human adrenal membranes, but it must be noted that such preparations were conceivably obtained from the entire adrenal gland, i.e., including both cortex and medulla.

3. Adrenocortical Cell Growth. The presence of OX2-Rs in adrenocortical cells not coupled to any secretory effect of orexins may suggest the alternative possibilities that either they are silent receptors or mediate a growth effect of these peptides. The latter appears to be the most convincing possibility in light of the following findings.

The systemic administration of orexins was found to increase the proliferation index in immature rat adrenal cortex and to lower it in enucleated glands at day 5 but not at day 8 of regeneration (Malendowicz et al., 2001b). Orexin-A (24-h exposure) increased the proliferation rate of cultured rat adrenocortical cells, but the effect was inversely correlated with the peptide concentration (maximum at 10^{-10} M, and minimum at 10^{-6} M). In contrast, orexin-B (10^{-8} or 10^{-6} M) decreased the proliferation rate. OX1-R immunoblockade reversed the proliferogenic effect of orexin-A, causing a small but significant decrease in growth. OX2-R immunoneutralization magnified the proliferogenic effect of orexin-A and abrogated the antiproliferogenic action of orexin-B (Spinazzi et al., 2005c). The proliferogenic effect of orexin-A (in the presence of OX2-R blockade) was annulled by the mitogen-activated protein kinase (MAPK) p42/p44 inhibitor PD98059, and the antiproliferogenic action of orexin-B was abrogated by the MAPK p38 inhibitor SB203580 (Spinazzi et al., 2005c). Thus, it was concluded that OX1-Rs and OX2-Rs mediate the proliferogenic and antiproliferogenic actions of orexins on rat adrenocortical cells through the activation of the MAPK p42/p44 and p38 cascades, respectively.

Different results were obtained in cultured human adrenocortical cells, in which both orexins (10^{-8} M) enhanced proliferative activity (Spinazzi et al., 2005b). Because orexin-B was more effective than orexin-A, it has been suggested that OX2-Rs mediate the growth-promoting action of orexins. Further investigations must be carried out to solve these discrepancies between rats and humans, also in light of findings showing an OX1-R-mediated antigrowth (proapoptotic) effect of orexins in human colon cancer- and neuroblastoma-derived cell lines as well as in CHO OX1-R-transfected cells (Rouet-Benzineb et al., 2004).

4. Catecholamine Secretion. Contrasting findings on the effects of orexins on adrenal-medulla secretion of

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

aspet

various mammalian species exist. Using the rat pheochromocytoma-derived PC12 cell line, Nanmoku et al. (2000) showed that both orexin-A and orexin-B (10^{-7} M) decreased either basal or pituitary adenylate cyclase-activating polypeptide-stimulated tyrosine hydroxylase expression, cAMP release, and dopamine secretion. Because PC12 cells displayed equal binding capacity for both orexins, these investigators suggested that orexins inhibited catecholamine synthesis via OX2-Rs negatively coupled to the AC-dependent cascade.

Orexin-A (10^{-10} M) was found to enhance TH activity and catecholamine synthesis (but not release) in cultured bovine adrenomedullary cells that expressed only OX1-R mRNA (Kawada et al., 2003). The effect was blocked by the PKC inhibitor staurosporin, making likely the possibility that orexins stimulate catecholamine synthesis in bovine adrenal medulla via OX1-Rs coupled to the PKC pathway.

Orexin-A (10^{-6} M) increased epinephrine and norepinephrine release from cultured pig adrenomedullary cells that possessed low OX1-R expression (Nanmoku et al., 2002). Orexin-A was also found to lower both basal and pituitary adenylate cyclase-activating polypeptide-stimulated cAMP production, thereby suggesting that orexins stimulate catecholamine secretion from pig adrenal medulla via OX1-Rs negatively coupled to the AC-dependent cascade.

Finally, human adrenal medulla slices did not exhibit any appreciable secretory response to orexins, despite the fact that they express both OX1-R and OX2-R mRNAs (Mazzocchi et al., 2001b) and that orexins exert a relevant catecholamine secretagogue action on pheochromocytoma slices (see section V.C.2.).

V. Involvement of Orexins in the Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

A. Stressful Conditions

Evidence suggests that orexins may modulate HPA axis response to different types of stress in the rat. The systemic administration of orexin-A and orexin-B (10 nmol/kg) magnified ACTH and glucocorticoid response to cold stress. Conversely, the response to ether stress was enhanced only by orexin-B (Nowak et al., 2000). Immobilization stress increased pp-orexin mRNA expression in the lateral hypothalamic area in juvenile rats (2 month-old), and cold stress evoked the same effect in adult animals (Ida et al., 2000).

These findings could indicate that orexins act as prostress agents, which is of great interest in light of the possible antistress effect of leptin (Bornstein, 1997; Heiman et al., 1997). The possibility of interactions between leptin and orexins at the hypothalamic level are supported by the demonstration that 1) neurons provided with leptin receptors innervated hypothalamic orexin-positive neurons (Funahashi et al., 2000), 2) leptin receptors were expressed in some orexin-positive neurons (Horvath et al., 1999), and 3) systemic leptin administration enhances pp-orexin mRNA in newborn rat hypothalamus (Yamamoto et al., 2000).

B. Adrenocortical Tumors

1. Orexin and Orexin Receptor Expression. Earlier studies did not report pp-orexin mRNA and orexin-A immunoreactivity in adrenocortical tumors (Arihara et al., 2000), but more recent investigations detected pp-orexin mRNA in 75% of cortisol-secreting adenomas, and measurable concentrations of orexin-A (12 ± 4 fmol/mg), but not orexin-B, in 70% of these tumors (Spinazzi et al., 2005b). ICC demonstrated that adrenocortical adenomas were provided with OX1-Rs, but not OX2-Rs (Blanco et al., 2002). Using real-time PCR and Western blotting, Spinazzi et al. (2005b) not only showed that all cortisol-secreting adenomas examined possessed both OX1-Rs and OX2-Rs but also that their mRNA was significantly up-regulated compared with that in normal adrenal cortex (114% and 63% higher).

2. Effects of Orexins on Secretion and Growth. Orexin-A concentration dependently increased basal cortisol production from freshly dispersed adenomatous cells, minimal and maximal effective concentrations being 10^{-10} and 10^{-8} M, respectively. Orexin-B was ineffective, which is consistent with the exclusive involvement of OX1-Rs (Spinazzi et al., 2005b). OX1-Rs were conceivably coupled to the AC/PKA cascade, because specific inhibitors of AC and PKA, but not of PLC and PKC, abolished the cortisol secretagogue effect of 10^{-8} M orexin-A. Spinazzi et al. (2005b) also demonstrated that both orexins (10^{-8} M) significantly enhanced the proliferative activity of cultured cortisol-secreting adenoma cells, thereby suggesting the involvement of OX2-Rs (see section IV.B.3.).

3. Conclusions. The effectiveness of orexins in eliciting both secretory and growth responses was markedly higher in cortisol-secreting adenomas than in normal adrenal cortex: the efficacy (percent increase elicited by the maximal effective concentration) of the secretagogue and proliferogenic effects of orexins were approximately 2-fold and 3- to 4-fold higher in adenomas (Spinazzi et al., 2005b). This observation accords well with the reported up-regulation of OX1-Rs and OX2-Rs in adenomas, and, along with the ability of adenomas to synthesize orexin-A, suggests that endogenous orexin system may be involved, through an autocrine-paracrine mechanism, in the pathogenesis of cortisol-secreting adrenal tumors.

C. Pheochromocytomas

1. Orexin and Orexin Receptor Expression. Although pp-orexin mRNA and orexin-A protein have been detected in ganglioneuroblastomas and neuroblastomas, they were not found in pheochromocytomas (Arihara et al., 2000). At variance, the expression of OX2-Rs but not of OX1-Rs, as mRNA and protein, has been demon-

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

PHARM REV

PHARMACOLOGICAL REVIEWS

strated in all benign human pheochromocytomas so far examined (Mazzocchi et al., 2001a; Blanco et al., 2002).

2. Effects of Orexins on Catecholamine Secretion. Mazzocchi et al. (2001a) showed that both orexin-A and orexin-B equipotently enhanced catecholamine secretion from pheochromocytoma quarters, with the maximal effective concentration being 10^{-8} M. Orexins (10^{-8} M) stimulated IP_3, but not cAMP, production from tumor slices, and catecholamine secretory response to orexins was abrogated by the inhibitors of PLC and PKC and unaffected by the inhibitors of AC and PKA. Taken together, these findings suggested that orexins stimulate pheochromocytoma secretion via OX2-Rs coupled to the PLC/PKC signaling cascade.

VI. Concluding Remarks

The preceding sections of this review have shown that in the 7 years elapsed from their discovery findings suggesting that orexins can play a potentially important role in the regulation of the function of the HPA axis have accumulated. However, several problems remain to be addressed, and we shall now take the opportunity to mention some issues that surely await further investigative effort.

Is the regulatory role of orexin on adrenal secretion relevant under physiological conditions? The bulk of evidence indicates that orexins stimulate CRH-ACTH secretion. The existence of orexinergic fibers reaching the PVN, which is provided with orexin receptors may easily account for the stimulating effect of orexin on CRH and AVP release but surely not for the activation of orexin receptors in the pituitary gland (see section III.B.). According to Nussdorfer (1996), if the tissue content of a regulatory peptide is 100 fmol/g, its 30% release will produce a local concentration of approximately 10^{-9} M. The orexin-A concentrations in rat and human hypothalamus range between 200 and 20 fmol/mg (Arihara et al., 2000; Spinazzi et al., 2005b); hence they could give rise to local orexin-A levels of approximately 2×10^{-6} and 2×10^{-7} M, that, via the hypothalamopituitary portal blood, could be capable of activating the OX1-Rs and OX2-Rs found in the pituitary gland. The physiological relevance of this central effect of orexins is indirectly confirmed by the blunted ACTH secretion occurring in hypothalamic orexin-deficient narcoleptic patients (Kok et al., 2002). Consistent experimental findings showed that orexins may enhance glucocorticoid secretion acting not only on the central branch of the HPA axis but also directly on adrenocortical cells that are provided with orexin receptors. However, the relevance of the latter mechanism of action of orexins remains questionable: in fact, 1) the level of circulating orexin-A in rat and humans does not exceed 15 and 2 pmol/l (Arihara et al., 2001; Jöhren et al., 2001, 2003); 2) minimal effective concentrations of orexin-A in eliciting in vitro glucocorticoid secretagogue effect are approximately 10^{-8} and 10^{-10} M in rats and humans (see section IV.B.2.); and 3) no measurable concentrations of orexins are present in normal adrenal glands (see section IV.A.1.), thereby ruling out the possibility of the involvement of an autocrine-paracrine mechanism of action. However, because fasting and hypoglycemia increase orexin concentrations in the hypothalamus (Sakurai et al., 1998; Cai et al., 1999, 2001; Karteris et al., 2005) and hypoglycemia enhances pancreatic release of orexins (Ouedraogo et al., 2003), it cannot be excluded that in these conditions the blood concentrations of orexins could reach levels capable of directly stimulating the secretion of adrenocortical cells. However, this possibility apparently conflicts the recent demonstration that fasting down-regulates orexin receptor expression in the rat adrenal glands (Karteris et al., 2005) (see section IV.A.2.). In this connection, we want to recall that leptin, which decreases food intake and body weight (Remesar et al., 1997; Mantzoros and Moschos, 1998; Ahima and Flier, 2000) and interacts with the orexin system at the hypothalamic level (see section V.A.), displays increased plasma concentrations in obese and hyperglycemic patients with Cushing's syndrome (Leal-Cerro et al., 1996; Blum et al., 1997; Weise et al., 1999). Because leptin inhibits glucocorticoid secretion (Bornstein et al., 1997; Glasow et al., 1998; Pralong et al., 1998; Glasow and Bornstein, 2000), the possibility awaits to be explored whether orexins together with leptin may take part in counterregulatory mechanisms, which, through the modulation of glucocorticoid secretion, are involved in the maintenance of glycemia and body weight homeostasis. Surely, in vivo investigations on this topic could take advantage of the use of animals in which endogenous orexin system is stably suppressed (e.g., orexin gene knockout animals). So far immunoblockade of orexin receptors with specific antibodies has been used in in vitro experiments (Spinazzi et al., 2005c; Ziolkowska et al., 2005), but this approach is unfeasible in long-term in vivo experiments. Furthermore, at present, only a selective not yet commercially available OX1-R nonpeptide antagonist (SB-334867) has been synthesized (Porter et al., 2001; Smart et al., 2001).

Are there functional interrelationships between adrenal and gonadal orexin systems? Orexin immunoreactivity has been detected in the rat testis (Mitsuma et al., 2000), orexin receptors were found in rat and human testis (Jöhren et al., 2001; Barreiro et al., 2004; Karteris et al., 2004), and orexin-A has been reported to enhance testosterone secretion both in vivo and in vitro in the rat (Barreiro et al., 2004). Although in the rat hypothalamus orexin expression is increased in proestrus and during pregnancy (see section III.A.1.), orexin receptor expression in adrenals appears to be down-regulated by estrogens and up-regulated by androgens (see section IV.A.2.). Further experiments are needed to ascertain whether the testis orexin system and gonadal hormones

Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

may account for the gender-related differences in orexin system expression in the HPA axis (see section IV.A.2.).

Is the orexin system involved in the pathophysiology of adrenal tumors? Cortisol-secreting adrenal adenomas were found not only to possess up-regulated orexin receptors but also to contain sizable amounts of orexin-A (12 fmol/mg), which could give rise to local concentrations (approximately 10^{-7} M) well above those able to elicit in vitro secretory and proliferative responses (see section V.B.). Despite the fact that the effects of orexin on catecholamine production from adrenal medulla are doubtful (see section IV.B.4.), orexins have been shown to stimulate catecholamine secretion from human pheochromocytomas (see section V.C.2.). Unfortunately, neither the orexin content of pheochromocytomas nor its possible effect on tumor cell growth has been studied. Additional investigations must be carried out to explore the appealing possibility that the orexin system plays a relevant role in the control of secretion and growth of adrenal tumors.

The answers to these questions, along with the development of potent and selective antagonists of orexin receptors, not only will increase our understanding of the physiology of the HPA axis but also could open new perspectives in the therapy of many diseases coupled to dysregulation of adrenal function.

Acknowledgments. We thank Alberta Coi for secretarial support and invaluable help in the provision of bibliographic items.

REFERENCES

Ahima RS and Flier JS (2000) Leptin. *Annu Rev Physiol* **62:**413–437.

Al-Barazanji KA, Wilson S, Baker J, Jessop DS, and Harbuz MS (2001) Central orexin-A activates hypothalamic-pituitary-adrenal axis and stimulates hypothalamic corticotropin releasing factor and arginine vasopressin neurones in conscious rats. *J Neuroendocrinol* **13:**421–424.

Ammoun S, Holmqvist T, Shariatmadari R, Oonk HB, Detheux M, Parmentier M, Åkerman KEO, and Kukkonen JP (2003) Distinct recognition of OX1 and OX2 receptors by orexin peptides. *J Pharmacol Exp Ther* **305:**507–514.

Arihara Z, Takahashi K, Murakami O, Totsune K, Sone M, Satoh F, Ito S, Hayashi Y, Sasano H, and Mouri T (2000) Orexin-A in the human brain and tumor tissues of ganglioneuroblastoma and neuroblastoma. *Peptides* **21:**565–570.

Arihara Z, Takahashi K, Murakami O, Totsune K, Sone M, Satoh F, Ito S, and Mouri T (2001) Immunoreactive orexin-A in human plasma. *Peptides* **22:**139–142.

Baker JR, Cardinal K, Bober C, Taylor MM, and Samson WK (2003) Neuropeptide W acts in brain to control prolactin, corticosterone and growth hormone release. *Endocrinology* **144:**2816–2821.

Barreiro ML, Pineda R, Navarro VM, Lopez M, Suominen JS, Pinilla L, Señaris R, Toppari J, Aguilar E, Dieguez C, et al. (2004) Orexin 1 receptor messenger ribonucleic acid expression and stimulation of testosterone secretion by orexin-A in rat testis. *Endocrinology* **145:**2297–2306.

Beuckmann CT and Yanagisawa M (2002) Orexins: from neuropeptides to energy homeostasis and sleep/wake regulation. *J Mol Med* **80:**329–342.

Blanco M, Garcia-Caballero T, Fraga M, Gallego R, Cuevas J, Forteza J, Beiras A, and Dieguez C (2002) Cellular localization of orexin receptors in human adrenal gland, adrenocortical adenomas and pheochromocytomas. *Regul Pept* **104:**161–165.

Blanco M, Lopez M, Garcia-Caballero T, Gallego R, Vazquez-Boquete A, Morel G, Señaris R, Casanueva F, Dieguez C, and Beiras A (2001) Cellular localization of orexin receptors in human pituitary. *J Clin Endocrinol Metab* **86:**1616–1619.

Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, et al. (1997) Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage and testosterone. *J Clin Endocrinol Metab* **82:**2904–2910.

Bornstein SR (1997) Is leptin a stress related peptide? *Nat Med* **3:**397.

Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, and Scherbaum WA (1997) Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* **46:**1235–1238.

Brunton PJ and Russell JA (2003) Hypothalamic-pituitary-adrenal responses to centrally administered orexin-A are suppressed in pregnant rats. *J Neuroendocrinol* **15:**633–637.

Cai XJ, Evans ML, Lister CA, Leslie RA, Arch JRS, Wilson S, and Williams G (2001) Hypoglycemia activates orexin neurons and selectively increases hypothalamic orexin-B levels: responses inhibited by feeding and possibly mediated by the nucleus of the solitary tract. *Diabetes* **50:**105–112.

Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JR, Tadayyon M, Clapham JC, Wilding J, and Williams G (1999) Hypothalamic orexin expression: modulation by blood glucose and feeding. *Diabetes* **48:**2132–2137.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, et al. (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98:**437–451.

Chen CT, Dun SL, Kwok EH, Dun NJ, and Chang JK (1999) Orexin A-like immunoreactivity in the rat brain. *Neurosci Lett* **260:**161–164.

Cluderay JE, Harrison DC, and Hervieu GJ (2002) Protein distribution of the orexin-2 receptor in the rat central nervous system. *Regul Pept* **104:**131–144.

Crawley JN and Corwin RL (1994) Biological actions of cholecystokinin. *Peptides* **15:**731–755.

Cutler DJ, Morris R, Sheridhar V, Wattam TA, Holmes S, Patel S, Arch JR, Wilson S, Buckingham RE, Evans ML, et al. (1999) Differential distribution of orexin-A and orexin-B immunoreactivity in the rat brain and spinal cord. *Peptides* **20:**1455–1470.

Dallman MF, Akana SF, Bhatnagar S, Bell ME, Choi S, Chu A, Horsley C, Levin N, Meijer O, Soriano LR, et al. (1999) Starvation: early signals, sensors and sequelae. *Endocrinology* **135:**4015–4023.

Date Y, Mondal MS, Matsukura S, Ueta Y, Yamashita H, Kaiya H, Kangawa K, and Nakazato M (2000) Distribution of orexin/hypocretin in the rat median eminence and pituitary. *Mol Brain Res* **76:**1–6.

Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, and Nakazato M (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci USA* **96:**748–753.

De Lecea L, Kilduff TS, Peyron C, Gao XB, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett FS, et al. (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA* **95:**322–327.

De Lecea L and Sutcliffe JG (1999) The hypocretins/orexins: novel hypothalamic neuropeptides involved in different physiological systems. *Cell Mol Life Sci* **56:**473–480.

Dyer CJ, Touchette KJ, Carroll JA, Allee GL, and Matteri RL (1999) Cloning of porcine prepro-orexin cDNA and effects of an intramuscular injection of synthetic porcine orexin-B on food intake in young pigs. *Domest Anim Endocrinol* **16:**145–148.

Engelmann M, Landgraf R, and Wotjak CT (2004) The hypothalamic-neurohypophyseal system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. *Front Neuroendocrinol* **25:**132–149.

Ferguson AV and Samson WK (2003) The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function. *Front Neuroendocrinol* **24:**141–150.

Funahashi H, Hori T, Shimoda Y, Mizushima H, Ryushi T, Katoh S, and Shioda S (2000) Morphological evidence for neural interactions between leptin and orexin in the hypothalamus. *Regul Pept* **92:**31–35.

Glasow A and Bornstein SR (2000) Leptin and the adrenal gland. *Eur J Clin Invest* **3:**39–45.

Glasow A, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP, and Bornstein SR (1998) Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. *J Clin Endocrinol Metab* **83:**4459–4466.

Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, and Flier JS (1997) Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* **138:**3859–3863.

Hervieu GJ, Cluderay JE, Harrison DC, Roberts JC, and Leslie RA (2001) Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience* **103:**777–797.

Hochól A, Albertin G, Nussdorfer GG, Spinazzi R, Ziolkowska A, Rucinski M, and Malendowicz LK (2004) Effects of neuropeptides B and W on the secretion and growth of rat adrenocortical cells. *Int J Mol Med* **14:**843–847.

Holmqvist T, Åkerman KEO and Kukkonen JP (2001) High specificity of human orexin receptors for orexins over neuropeptide Y and other neuropeptides. *Neurosci Lett* **305:**177–180.

Holmqvist T, Johansson L, Östman M, Ammount S, Åkerman KEO, and Kukkonen JP (2005) OX1 orexin receptors couple to adenylyl cyclase regulation via multiple mechanisms. *J Biol Chem* **280:**6570–6579.

Horvath T, Diano S, and Van den Pol AN (1999) Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* **19:**1072–1087.

Hungs M and Mignot E (2001) Hypocretin/orexin, sleep and narcolepsy. *Bioessays* **23:**397–408.

Ida T, Nakahara K, Murakami T, Hanada R, Nakazato M, and Murakami N (2000) Possible involvement of orexin in the stress reaction in rats. *Biochem Biophys Res Commun* **270:**318–323.

Jaszberényi M, Bujdosó E, Pataki I, and Telegdy G (2000) Effects of orexins on the hypothalamic-pituitary-adrenal system. *J Neuroendocrinol* **12:**1174–1178.

Jaszberényi M, Bujdosó E, and Telegdy G (2001) The role of neuropeptide Y in orexin-induced hypothalamic-pituitary-adrenal activation. *J Neuroendocrinol* **13:**438–441.

Jöhren O, Brüggemann N, Dendorfer A, and Dominiak P (2003) Gonadal steroids differentially regulate the messenger ribonucleic acid expression of pituitary orexin type 1 receptors and adrenal orexin type 2 receptors. *Endocrinology* **144:**1219–1225.

Jöhren O, Brüggemann N, and Dominiak P (2004) Orexins (hypocretins) and adrenal function. *Horm Metab Res* **36:**370–375.

Jöhren O, Neidert SJ, Kummer M, Dendorfer A, and Dominiak P (2001) Prepro-

PHARM REV
PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. *Endocrinology* **142:**3324–3331.
Kane JK, Tanaka H, Parker SL, Yanagisawa M, and Li MD (2000) Sensitivity of orexin-A binding to phospholipase C inhibitors, neuropeptide Y and secretin. *Biochem Biophys Res Commun* **272:**959–965.
Kanenishi K, Ueno M, Momose S, Kuwabara H, Tanaka H, Sato C, Kobayashi T, Hino O, Sakamoto H, and Hata T (2004) Prepro-orexin mRNA expression in the rat brain is increased during pregnancy. *Neurosci Lett* **368:**73–77.
Karteris E, Chen J, and Randeva HS (2004) Expression of human prepro-orexin and signaling characteristics of orexin receptors in the male reproductive system. *J Clin Endocrinol Metab* **89:**1957–1962.
Karteris E, Machado RJ, Chen J, Zervou S, Hillhouse EW, and Randeva HS (2005) Food deprivation differentially modulates orexin receptor expression and signaling in rat hypothalamus and adrenal cortex. *Am J Physiol* **288:**E1089–E1100.
Karteris E, Randeva HS, Grammatopoulos DK, Jaffe RB, and Hillhouse EW (2001) Expression and coupling characteristics of the CRH and orexin type 2 receptors in human fetal adrenals. *J Clin Endocrinol Metab* **86:**4512–4519.
Kastin AJ and Akerstrom V (1999) Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. *J Pharmacol Exp Ther* **289:**219–223.
Kawada Y, Ueno S, Asayama K, Tsutsui M, Utsunomiya K, Toyohira Y, Morisada N, Tanaka K, Shirahata A, and Yanagihara N (2003) Stimulation of catecholamine synthesis by orexin-A in bovine adrenal medullary cells through orexin receptor 1. *Biochem Pharmacol* **66:**141–147.
Kok SW, Roelfsema F, Overeem S, Lammers GJ, Strijers RL, Frölich M, Meinders AE, and Pijl H (2002) Dynamics of the pituitary-adrenal ensemble in hypocretin-deficient narcoleptic humans: blunted basal adrenocorticotropin release and evidence for normal time-keeping by the master pacemaker. *J Clin Endocrinol Metab* **87:**5085–5091.
Khorooshi RMH and Klingenspor M (2005) Neuronal distribution of melanin-concentrating hormone, cocaine- and amphetamine-regulated transcript and orexin B in the brain of the Djungarian hamster (*Phodopus sungorus*). *J Chem Neuroanat* **29:**137–148.
Kreegipuu A, Blom N, and Brunak S (1999) PhosphoBase, a database of phosphorylation sites: release 2.0. *Nucleic Acids Res* **27:**237–239.
Krysiak R, Obuchowicz E, and Herman ZS (1999) Interactions between the neuropeptide Y system and the hypothalamic-pituitary-adrenal axis. *Eur J Endocrinol* **140:**130–136.
Kukkonen JP, Holmqvist T, Ammoun S, and Åkerman KEO (2002) Functions of the orexinergic/hypocretinergic system. *Am J Physiol* **283:**C1567–C1591.
Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, and Yamashita H (2000) Centrally administered orexin/hypocretin activates HPA axis in rats. *Neuroreport* **11:**1977–1980.
Larsson KP, Peltonen HM, Bart G, Louhivuori LM, Penttonen A, Antikainen M, Kukkonen JP, and Åkerman KEO (2005) Orexin-A-induced Ca^{2+} entry: evidence for involvement of TRPC channels and protein kinase C regulation. *J Biol Chem* **280:**1771–1781.
Leal-Cerro A, Considine RV, Peino R, Venegas E, Astorga R, Casanueva FF, and Dieguez C (1996) Serum immuno-reactive leptin levels are increased in patients with Cushing's syndrome. *Horm Metab Res* **28:**711–713.
Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, De Jong PJ, Nishino S, and Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98:**365–376.
Lopez M, Señaris R, Gallego R, Garcia-Caballero T, Lago F, Seoane L, Casanueva F, and Dieguez C (1999) Orexin receptors are expressed in the adrenal medulla of the rat. *Endocrinology* **140:**5991–5994.
Lopez M, Seoane L, Garcia MC, Lago F, Casanueva FF, Señaris R, and Dieguez C (2000) Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. *Biochem Biophys Res Commun* **269:**41–45.
Lopez M, Seoane L, Señaris RM, and Dieguez C (2001) Prepro-orexin mRNA levels in the rat hypothalamus and orexin receptor mRNA levels in the rat hypothalamus and adrenal gland are not influenced by the thyroid status. *Neurosci Lett* **300:**171–175.
Lu XY, Bagnol D, Burke S, Akil H, and Watson SJ (2000) Differential distribution and regulation of OX1 and OX2 orexin/hypocretin receptor messenger RNA in the brain upon fasting. *Horm Behav* **37:**335–344.
Lund PE, Shariatmadari R, Uustare A, Detheux M, Parmentier M, Kukkonen JP, and Åkerman KEO (2000) The orexin OX1 receptor activates a novel Ca^{2+} influx pathway necessary for coupling to phospholipase C. *J Biol Chem* **275:**30806–30812.
Malendowicz LK, Hochól A, Ziolkowska A, Nowak M, Gottardo L, and Nussdorfer GG (2001a) Prolonged orexin administration stimulates steroid-hormone secretion, acting directly on the rat adrenal gland. *Int J Mol Med* **7:**401–404.
Malendowicz LK, Jedrzejczak N, Belloni AS, Trejter M, Hochól A, and Nussdorfer GG (2001b) Effects of orexins A and B on the secretory and proliferative activity of immature and regenerating rat adrenal glands. *Histol Histopathol* **16:**713–717.
Malendowicz LK, Nowak M, Gottardo L, Tortorella C, Majchrzak M, and Nussdorfer GG (2001c) Cholecystokinin stimulates aldosterone secretion from dispersed rat zona glomerulosa cells, acting through cholecystokinin receptors 1 and 2 coupled with the adenylate cyclase-dependent cascade. *Endocrinology* **142:**4251–4255.
Malendowicz LK, Spinazzi R, Majchrzak M, Nowak M, Nussdorfer GG, Ziolkowska A, Macchi C, and Trejter M (2003) Effects of prolonged cholecystokinin administration on rat pituitary-adrenocortical axis: role of the CCK receptor subtypes 1 and 2. *Int J Mol Med* **12:**903–909.
Malendowicz LK, Tortorella C, and Nussdorfer GG (1999a) Orexins stimulate corticosterone secretion of rat adrenocortical cells, through the activation of the adenylate cyclase-dependent signaling cascade. *J Steroid Biochem Mol Biol* **70:**185–188.
Malendowicz LK, Tortorella C, and Nussdorfer GG (1999b) Acute effects of orexins A and B on the rat pituitary-adrenocortical axis. *Biomed Res* **20:**301–304.
Mantzoros CS and Moschos SJ (1998) Leptin: in search of role(s) in human physiology and pathophysiology. *Clin Endocrinol* (*Oxford*) **49:**551–567.
Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, and Elmqvist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* **435:**6–25.
Matsumura K, Tsuchihashi T, and Abe I (2001) Central orexin-A augments sympathoadrenal outflow in conscious rabbits. *Hypertension* **37:**1382–1387.
Mazzocchi G, Malendowicz LK, Aragona F, Rebuffat P, Gottardo L, and Nussdorfer GG (2001a) Human pheochromocytomas express orexin receptor type 2 gene and display an in vitro secretory response to orexins A and B. *J Clin Endocrinol Metab* **86:**4818–4821.
Mazzocchi G, Malendowicz LK, Aragona F, Spinazzi R, and Nussdorfer GG (2004) Cholecystokinin (CCK) stimulates aldosterone secretion from human adrenocortical cells via CCK2 receptors coupled to the adenylate cyclase/protein kinase A signaling cascade. *J Clin Endocrinol Metab* **89:**1277–1284.
Mazzocchi G, Malendowicz LK, Gottardo L, Aragona F, and Nussdorfer GG (2001b) Orexin A stimulates cortisol secretion from human adrenocortical cells through activation of the adenylate cyclase-dependent signaling cascade. *J Clin Endocrinol Metab* **86:**778–782.
Mazzocchi G, Rebuffat P, Ziolkowska A, Rossi GP, Malendowicz LK, and Nussdorfer GG (2005) G protein receptors (GPR) 7 and 8 are expressed in human adrenocortical cells and their endogenous ligands neuropeptides B and W enhance cortisol secretion by activating adenylate cyclase- and phospholipase C-dependent signaling cascades. *J Clin Endocrinol Metab* **90:**3466–3471.
McGranaghan PA and Piggins HD (2001) Orexin A-like immunoreactivity in the hypothalamus and thalamus of the Syrian hamster (*Mesocricetus auratus*) and Siberian hamster (*Phodopus sungorus*), with special reference to circadian structures. *Brain Res* **904:**234–244.
Meier CA (1998) Orexins and anorexins: thoughts for food. *Eur J Endocrinol* **139:**148–149.
Mignot E (2001) A commentary on the neurobiology of the hypocretin/orexin system. *Neuropsychopharmacology* **25:**S5–S13.
Mitsuma T, Hirooka Y, Kayama M, Mori Y, Yokoi Y, Rhue N, Ping J, Izumi M, Ikai R, Adachi K, et al. (2000) Radioimmunoassay for orexin A. *Life Sci* **66:**897–904.
Moreno G, Perello M, Gaillard RC, and Spinedi E (2005) Orexin A stimulates hypothalamic-pituitary-adrenal (HPA) axis function, but not food intake, in the absence of full hypothalamic NPY-ergic activity. *Endocrine* **26:**99–106.
Nakabayashi M, Suzuki T, Takahashi K, Totsune K, Muramatsu Y, Kaneko C, Date F, Takeyama J, Darnel AD, Moriya T, et al. (2003) Orexin-A expression in human peripheral tissues. *Mol Cell Endocrinol* **205:**43–50.
Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, and Goto K (1999) Distribution of orexin neurons in the adult rat brain. *Brain Res* **827:**243–260.
Nanmoku T, Isobe K, Sakurai T, Yamanaka A, Takekoshi K, Kawakami Y, Goto K, and Nakai T (2002) Effects of orexin on cultured porcine adrenal medullary and cortex cells. *Regul Pept* **104:**125–130.
Nanmoku T, Isobe K, Sakurai T, Yamanaka A, Takekoshi K, Kawakami Y, Ishii K, Goto K, and Nakai T (2000) Orexins suppress catecholamine synthesis and secretion in cultured PC12 cells. *Biochem Biophys Res Commun* **274:**310–315.
Nishino S, Ripley B, Overeem S, Lammers GJ, and Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* **355:**39–40.
Nowak M, Hochól A, Tortorella C, Jedrzejczak N, Ziolkowska A, Nussdorfer GG, and Malendowicz LK (2000) Modulatory effects of orexins on the function of rat pituitary-adrenocortical axis under basal and stressful conditions. *Biomed Res* **21:**89–93.
Nussdorfer GG (1996) Paracrine control of adrenal cortical function by medullary chromaffin cells. *Pharmacol Rev* **48:**495–530.
Nussdorfer GG, Rossi GP, Malendowicz LK, and Mazzocchi G (1999) Autocrine-paracrine endothelin system in the physiology and pathology of steroid secreting tissues. *Pharmacol Rev* **51:**403–438.
Nussdorfer GG, Spinazzi R, and Mazzocchi G (2005) Cholecystokinin (CCK) and adrenal-cortex secretion. *Vitam Horm* **71:**433–453.
Ouedraogo R, Näslund E, and Kirchgessner AL (2003) Glucose regulates the release of orexin-A from the endocrine pancreas. *Diabetes* **52:**111–117.
Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, et al. (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6:**991–997.
Porkka-Heiskanen T, Kalinchuk A, Alanko L, Huhtaniemi I, and Stenberg D (2004) Orexin A and B levels in the hypothalamus of female rats: the effects of the estrous cycle and age. *Eur J Endocrinol* **150:**737–742.
Porter RA, Chan WN, Coulton S, Johns A, Hadley MS, Widdowson K, Jerman JC, Brough SJ, Coldwell M, Smart D, et al. (2001) 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. *Bioorg Med Chem Lett* **11:**1907–1910.
Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, and Gaillard RC (1998) Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. *Endocrinology* **139:**4264–4268.
Randeva HS, Karteris E, Grammatopoulos DK, and Hillhouse EW (2001) Expression of orexin-A and functional orexin type 2 receptors in the human adult adrenals: implications for adrenal function and energy homeostasis. *J Clin Endocrinol Metab* **86:**4808–4813.
Remesar X, Rafecas I, Fernandez-Lopez JA, and Alemany M (1997) Leptin. *Med Res Rev* **17:**225–234.
Renshaw D and Hinson JP (2001) Neuropeptide Y and the adrenal gland: a review. *Peptides* **22:**429–438.
Rouet-Benzineb P, Rouyer-Fessard C, Jarry A, Avondo V, Pouzet C, Yanagisawa M, Laboisse C, Laburthe M, and Voisin T (2004) Orexins acting at native OX1 receptor in colon cancer and neuroblastoma cells or at recombinant OX1 receptor suppress cell growth by inducing apoptosis. *J Biol Chem* **279:**45875–45886.
Russell SH, Small CJ, Dakin CL, Abbott CR, Morgan DGA, Ghatei MA, and Bloom SR (2001) The central effects of orexin-A in the hypothalamic-pituitary-adrenal axis in vivo and in vitro in male rats. *J Neuroendocrinol* **13:**561–566.
Sakurai T (1999) Orexin and orexin receptors: implication in feeding behaviour. *Regul Pept* **85:**25–30.

PHARM REV
PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012

Sakurai T (2005) Reverse pharmacology of orexin: from an orphan GPCR to integrative physiology. *Regul Pept* **126:**3–10.
Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, et al. (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92:**573–585.
Sakurai T, Moriguchi T, Furuya K, Kajiwara N, Nakamura T, Yanagisawa M, and Goto K (1999) Structure and function of human prepro-orexin gene. *J Biol Chem* **274:**17771–17776.
Samson WK and Resch ZT (2000) The hypocretin/orexin story. *Trends Endocrinol Metab* **11:**257–262.
Samson WK and Taylor MM (2001) Hypocretin/orexin suppresses corticotroph responsiveness in vitro. *Am J Physiol* **281:**R1140–R1145.
Samson WK, Taylor MM, Follwell M, and Ferguson AV (2002) Orexin actions in hypothalamic paraventricular nucleus: physiological consequences and cellular correlates. *Regul Pept* **104:**97–103.
Siegel JM, Moore R, Thannickal T, and Nienhuis R (2001) A brief history of hypocretin/orexin and narcolepsy. *Neuropsychopharmacology* **25:**S14–S20.
Smart D, Jerman JC, Brough SJ, Rushton SL, Murdock PR, Jewitt F, Elshourbagy NA, Ellis CE, Middlemiss DN, and Brown F (1999) Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. *Br J Pharmacol* **128:**1–3.
Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA, and Jerman JC (2001) SB-334867-A: the first selective orexin-1 receptor antagonist. *Br J Pharmacol* **132:**1179–1182.
Spinazzi R, Andreis PG, and Nussdorfer GG (2005a) Neuropeptide-Y and Y-receptors in the autocrine-paracrine regulation of adrenal gland under physiological and pathophysiological conditions. *Int J Mol Med* **15:**3–13.
Spinazzi R, Rucinski M, Neri G, Malendowicz LK, and Nussdorfer GG (2005b) Prepro-orexin and orexin receptors are expressed in cortisol-secreting adrenocortical adenomas and orexins stimulate in vitro cortisol secretion and growth of tumor cells. *J Clin Endocrinol Metab* **90:**3544–3549.
Spinazzi R, Ziolkowska A, Neri G, Nowak M, Rebuffat P, Nussdorfer GG, Andreis PG and Malendowicz LK (2005c) Orexins modulate the growth of cultured rat adrenocortical cells, acting through type 1 and type 2 receptors coupled to the MAPK p42/p44- and p38-dependent cascades. *Int J Mol Med* **15:**847–852.
Stricker-Krongrad A and Beck B (2002) Modulation of hypothalamic hypocretin/orexin mRNA expression by glucocorticoids. *Biochem Biophys Res Commun* **296:**129–133.
Taheri S and Bloom S (2001) Orexins/hypocretins: waking up the scientific world. *Clin Endocrinol (Oxford)* **54:**421–429.
Taylor MM and Samson WK (2003) The other side of the orexins: endocrine and metabolic actions. *Am J Physiol* **284:**E13–E17.
Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, and Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27:**469–474.
Trivedi P, Yu H, Mac Neil DJ, Van der Ploeg LH, and Guan XM (1998) Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett* **438:**71–75.
Van den Pol AN, Gao XB Obrietan K, Kilduff TS and Belousov AB (1998) Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J Neurosci* **18:**7962–7971.
Wauters M, Considine RV, and Van Gaal LF (2000) Human leptin: from an adipocyte hormone to an endocrine mediator. *Eur J Endocrinol* **143:**293–311.
Weise M, Abad V, Considine RV, Nieman L, and Rother KI (1999) Leptin secretion in Cushing's syndrome: preservation of diurnal rhythm and absent response to corticotropin-releasing hormone. *J Clin Endocrinol Metab* **84:**2075–2079.
Willie JT, Chemelli RM, Sinton CM, and Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci* **24:**429–458.
Wolf G (1998) Orexins: a newly discovered family of hypothalamic regulators of food intake. *Nutr Rev* **56:**172–189.
Yamamoto Y, Ueta Y, Hara Y, Serino R, Nomura M, Shibuya I, Shirahata A, and Yamashita H (2000) Postnatal development of orexin/hypocretin in rats. *Mol Brain Res* **78:**108–119.
Zhang S, Blache D, Vercoe PE, Adam CL, Blackberry MA, Findlay PA, Eidne KA, and Martin GB (2005) Expression of orexin receptors in the brain and peripheral tissues of the male sheep. *Regul Pept* **124:**81–87.
Ziolkowska A, Spinazzi R, Albertin G, Nowak M, Malendowicz LK, Tortorella C, and Nussdorfer GG (2005) Orexins stimulate glucocorticoid secretion from cultured rat and human adrenocortical cells, exclusively acting via the OX1 receptor. *J Steroid Biochem Mol Biol* **96:**423–429.

PHARM REV
PHARMACOLOGICAL REVIEWS
aspet
Downloaded from pharmrev.aspetjournals.org by guest on June 15, 2012